Targeting the Limitless Replicative Potential of Cancer: The Telomerase/Telomere Pathway

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Abstract

The maintenance of telomeric DNA underlies the ability of tumors to possess unlimited replicative potential, one of the hallmarks of cancer. Telomere length and structure are maintained by the reverse transcriptase telomerase and a multiprotein telomere complex termed shelterin. Telomerase activity is elevated in the vast majority of tumors, and telomeres are critically shortened in tumors versus normal tissues, thus providing a compelling rationale to target the telomerase/telomere pathway for broad-spectrum cancer therapy. This strategy is supported by a variety of genetic-based target validation studies. Both telomerase inhibitors and telomere interactive molecules have shown stand-alone antitumor activity at nontoxic doses against a variety of human tumor xenografts in mice. These translational advances have resulted in the first antitumor agent, the oligonucleotide-based GRN163L targeting the telomerase RNA template, entering clinical evaluation. Additional translational approaches, such as targeting telomeres using G-quadruplex ligands, should result in antitumor agents, such as RHPS4, entering the clinic in the near future. These prototype trials will be extremely informative in determining the role of the telomerase/telomere pathway in clinical oncology and, moreover, whether drugs targeting the unlimited replicative potential of cancer will find a place in cancer chemotherapy.

Background

Current cancer drug development is largely focused on combating the major phenotypical features of cancer [i.e., uncontrolled growth (through inactivation of tumor suppressors, activation of oncopgenes, and evasion of apoptosis)], unlimited replicative potential, formation of new blood vessels (angiogenesis), and tissue invasion and metastatic spread; ref. 1]. In recent years, this strategy has resulted in some notable success stories with new approved molecularly targeted drugs that have made a significant effect in lengthening the survival of cancer sufferers (e.g., imatinib in chronic myelogenous leukemia, trastuzumab in breast cancer, and bevacizumab in colorectal cancer; ref. 2).

The Relevance of the Telomere Maintenance Pathway to Cancer

Validation of a cancer target encompasses information on the prevalence and role of the target or pathway in human cancer, whether modulation of the target or pathway in preclinical model systems results in a robust anticancer phenotype (e.g., growth inhibition, induction of apoptosis or prevention of angiogenesis, migration, or invasion), and ultimately whether small-molecule or biological modulators provide a benefit to cancer patients (2). Pioneering studies done over the past 2 decades have shown that one of the above-described and to date therapeutically untapped phenotypic hallmarks of cancer, unlimited replicative potential, is intimately related to the maintenance of telomeres, repetitive TTAGGG sequences of DNA located on the ends of human chromosomes. Whereas mortal cells shorten their telomeres during each round of replication due to the “end-replication problem,” in tumor cells telomere length is stabilized mainly via reactivation of the reverse transcriptase termed telomerase, composed of a RNA component (htTR or hTERC) and a catalytic protein (htERT; see ref. 3 for a recent review). Moreover, the introduction of hTERT into normal human cells extends their life span (4) and hTERT (along with two oncopgenes, large T and H-ras) expression results in the direct tumorigenic conversion of normal human epithelial and fibroblast cells (5). Other studies, however, suggest that telomerase may also play a role in maintaining telomeres in some normal human cells (6) and may possess additional roles to telomere maintenance, such as regulation of chromatin state and DNA damage responses, as cells lacking hTERT possessed a diminished capacity for DNA double-strand break repair and fragmented chromosomes (7). In addition, in some tumors (particularly those of neuroepithelial and mesenchymal origin), telomeres are maintained by an alternative lengthening of telomere mechanism (8). In patients with glioblastoma multiforme tumors, alternative lengthening of telomere is associated with mutant TP53, as regulation of chromatin state and DNA damage responses, as cells lacking hTERT possessed a diminished capacity for DNA double-strand break repair and fragmented chromosomes (7). In addition, in some tumors (particularly those of neuroepithelial and mesenchymal origin), telomeres are maintained by an alternative lengthening of telomere mechanism (8). In patients with glioblastoma multiforme tumors, alternative lengthening of telomere is associated with mutant TP53, and a favorable prognosis (9); characteristics of alternative lengthening of telomere cells are alternative lengthening of telomere–associated promyelocytic leukemia bodies and circular extrachromosomal telomeric DNA (telomeric t circles, which require Xrcc3 and Nbs1 for their production; ref. 10).
Finally, a group (six to date) of telomere-associated proteins, collectively termed shelterin, comprising TRF1 and TRF2 that bind to double-stranded telomeric DNA, POT1 that binds the single-stranded 3’ G-rich overhang, and three interconnecting proteins (TIN2, TPP1, and RAP1), acts to shape and safeguard telomeres (Fig. 1; refs. 11, 12).

The interest in telomerase and telomeres in a cancer therapeutics context has emerged from two main observations: first, telomerase activity, typically using a PCR-based assay, telomeric repeat amplification protocol, is elevated in the great majority (≈85%) of tumor types in comparison with normal tissues, and second, telomeres are generally shorter in tumors than in corresponding normal tissues (see refs. 13, 14 for reviews). Furthermore, several studies provide genetic validation of telomerase as an anticancer target in that inactivation of hTR and/or hTERT by dominant-negative mutants or antisense strategies resulted in an inhibition of tumor cell proliferation. For example, transfection of a dominant-negative mutant of hTERT into cancer cell lines of differing initial telomere length resulted in growth inhibition but this occurred with a different lag time dependent on this initial telomere length (15). Such studies supported the view that, following inhibition of telomerase, telomeres needed to be gradually eroded down to a critical length before any phenotypic effect occurred. Hence, small-molecule mimics of this effect would be best suited to the treatment of tumors possessing very short telomeres.

Subsequent studies, however, targeting components of telomerase or telomeres/shelterin have shown that much more rapid apoptosis or induction of senescence effects can occur. For example, antisense oligonucleotide-mediated inhibition of hTERT, but not hTERC, caused a rapid reduction in cell growth and induction of apoptosis without telomere shortening in human prostate cancer cells (16). Second, the introduction of mutant template telomerase RNA into human tumor cells rapidly decreased cell viability and increased apoptosis, possibly via a telomere uncapping mechanism and triggering a DNA damage response (17, 18). Third, ribozymes targeting murine telomerase RNA induced antitumor effects, including

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**Fig. 1.** The telomerase/telomere pathway and main points of possible therapeutic intervention. Pathway modulators may result in either mainly telomere erosion (e.g., BIBR1532) resulting in relatively slow phenotypic anticancer effects or predominantly telomere uncapping (e.g., RHPS4) producing more rapid anticancer effects. Agents such as GRN163L probably mainly affect telomere erosion but also seem to induce telomere uncapping.
reducing melanoma tumor invasion and metastases in vivo; these studies also showed that telomerase activity may control the glycolytic pathway (19, 20). Finally, other genetic and biochemical studies suggest that targeting components of shelterin, such as TRF2 (21) or POT1 (12, 22, 23), or exposing the telomere 3’ overhang (24) can induce apoptosis or telomere-initiated senescence and thereby activate a DNA damage checkpoint response (25).

Because telomerase activity is relatively high in many tumors and telomeres are generally shorter in tumors than normal tissues, taken together with the above-described genetic-based validation studies, provide a compelling argument to suggest that the telomerase/telomere pathway is a well-validated target at the preclinical level. Therefore, appropriate modulators should be tested in patients.

### Clinical Translational Advances

As shown in Fig. 1, the burgeoning knowledge about the structure of telomeres and the roles of various proteins involved in telomere maintenance provides several possible targets for modulation. Some of these, such as modulating the interaction of TRF2 or POT1 with telomeres, may hold promise as cancer drugs but, as yet, have not been described. To date, two areas have received major drug discovery attention: first, the targeting of components of telomerase (e.g., hTR RNA template or hTERT protein), and second, the targeting of telomeres (e.g., G-quadruplex ligands that induce the formation of guanine-rich telomeres to stably fold into four-stranded G-quadruplex structures, thereby adversely affecting telomere function).

Although, as described above, telomerase represents an attractive cancer target, surprisingly few potent small-molecule inhibitors have been discovered to date. It is unclear whether this is because of a general lack of interest in pursuing this target within the pharmaceutical industry (perhaps because of the phenotypic lag arguments described above) or because the enzyme is not particularly tractable to the main current drug discovery approach using high-throughput screening of large compound libraries resulting in few chemical hits or leads emerging. One small-molecule nonnucleoside inhibitor of hTERT, BIBR1532, has been studied in preclinical models, including in vivo (26, 27). BIBR1532 inhibited telomerase in cell-free assays with an IC50 of 93 nmol/L and was shown to be a mixed-type noncompetitive inhibitor with a drug binding site distinct from the sites for deoxyribonucleotides and the DNA primer. Cell-based studies with BIBR1532 revealed a significant phenotypic lag, typically of ~100 days, between the onset of telomerase inhibition and cellular growth arrest, hence mimicking the above-described studies with dominant-negative hTERT. Antitumor effects were demonstrable in vivo in mice following inoculation of HT1080 fibrosarcoma cells that had been previously exposed to the compound for 100 days in vitro (and leading to a reduction in telomere length to 1.6 kb).

An oligonucleotide-based molecule that acts as a telomerase RNA template antagonist, GRN163L, represents the most advanced antitelomerase therapeutic. Phase I clinical trials have been initiated in patients with either chronic lymphocytic leukemia or solid tumors. GRN163L is a more potent derivative of GRN163, a 13-mer thio-phosphoramidate oligonucleotide complementary to the RNA template of hTR (28). Although cell-based studies with GRN163 generally showed a phenotypic lag of at least a few weeks before the onset of exposure and growth inhibition (e.g., ref. 28), the addition of a 5’ lipid (palmitate) moiety in GRN163L increased potency and resulted in a more rapid loss of telomeres and inhibition of cell growth (29). Furthermore, in vivo inhibition of tumor growth by GRN163L has been reported in mice in A549 human lung cancer metastases treated with 15 mg/kg thrice weekly for 3 consecutive weeks (30). Antitumor activity, observable from 2 to 3 weeks of thrice-weekly dosing, has also been reported in mice bearing orthotopic MDA-MB-231 human breast cancer xenografts (31).

Over the past decade, many chemical classes of G-quadruplex ligands have been described, the most potent of which, telomestatin, inhibits telomerase in the telomeric repeat amplification protocol assay at low nanomolar concentrations (32). Other compounds have been rationally designed based on the crystal structure of parallel quadruplexes from human telomeric DNA (33, 34). Several reduce the growth of various cancer cell lines in vitro after 1 to 2 weeks of exposure to low micromolar concentrations, often accompanied by cellular senescence (34–37), apoptosis (32, 36), and/or chromosomal fusions/anaphase bridges (32, 35, 36). Several G-quadruplex ligands have exhibited antitumor activity in vivo at nonacute toxic doses in mice bearing various human tumor xenografts: the 3,6,9-trisubstituted acridine BRACO19 in combination with paclitaxel against vulval carcinoma (34) or as monotherapy against DU145 prostate cancer (38) or UIX138L uterine cancer (39), the cationic porphyrin TMPyP4 against PC3 prostate cancer (40), telomestatin against U937 acute myelomonocytic leukemia (41), and the pentacyclic acridine RHP54 against CG5 breast cancer (42). In general, antitumor effects were apparent relatively rapidly following the onset of drug treatment, typically after ~10 days (34, 38–42). A consistent mechanism of action schema is emerging involving displacement of POT1 and TRF2 from telomeres and erosion of 3’ single-stranded telomeric overhangs (G-tails) with activation of a DNA damage response [e.g., reported with telomestatin (32, 43, 44) and RHP54 (42)]. Such findings suggest that the G-quadruplex ligands can induce relatively rapid telomere uncapping and may provide an explanation as to why reductions in telomere length are not always observed in cells exposed to this class of compound. The cell-based effects (senescence, apoptosis, activation of a DNA damage response including γH2AX phosphorylation, etc.) are also consistent with modulation of the currently known biological roles of POT1 (23) and TRF2 (21). Finally, measurements of POT1 or TRF2 displacement from telomeres or γH2AX phosphorylation may provide sensitive and appropriate biomarkers of target modulation in clinical trials. At least some of the G-quadruplex ligands, such as RHP54, are expected to enter clinical trials in the near future.

### Concluding Remarks

The telomerase/telomere field has come a long way in a relatively short time and is now at a particularly exciting juncture, as the first telomerase inhibitor, GRN163L, undergoes early clinical development. Other small-molecule modulators...
of telomerase/telomere biology, notably G-quadruplex ligands such as RHP54, are in preclinical development and are anticipated to enter the clinic in the near future. These clinical studies will be extremely informative in determining whether the positive anticancer findings from various genetic-based preclinical validation studies are borne out in cancer patients. Furthermore, based on the high prevalence of elevated telomerase and/or shortened telomeres in multiple tumor types in comparison with normal tissue counterparts, targeting telomerase/telomeres could be applicable to the treatment of a broad-spectrum of cancers, most likely in combination with existing chemotherapeutic drugs. Although there is relatively little to guide rational clinical combinations of antitelomerase/telomere therapeutics with other drugs at present (and although dose-limiting toxicities remain unknown), the association of telomerase/telomeres with DNA repair, particularly double-strand break repair, pathways is suggestive of this new class of agent being usefully combined with cytotoxics that induce DNA damage (e.g., platin and topoisomerase inhibitors) or ionizing radiation.

References

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